

Simultaneous Recovery of Pectin and Colorants from Solid Agro-wastes Formed in Processing of Colorful Berries

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Extraction of pectic substances from solid agro-wastes and the application of recovered pectin in the food, pharmaceutical and cosmetic industry can significantly contribute to a more economic and environmentally sound agro-industrial production. Thus investigation of the physicochemical properties of extracted pectic substances seems important not only from human health preservation considerations, but their advantageous properties can be confirmed for the possible manufacturers, potential processing, as well. Therefore, in this work pectic substances were extracted from red currant, black currant, raspberry, blackberry and elderberry press residues by hot water and the composition, antioxidant activity, total phenol, anthocyanin content and the color coordinates of pectins were determined. The results show that the pectin colors fall in the range between reddish purple (black currant and elderberry) and yellow (citrus and apple). Moreover some of the pectins in a powder form have different color coordinates than in the form of aqueous solutions or gels. This might be very important when the pectins are selected for different production processes. The anthocyanin content of pectin preparations is lower than the values of fruit juices. It can be concluded that the colorants found

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in pectin preparations belong to the group of phenolics and have adequate antioxidant capacity, which is extremely beneficial for human health. As a summary it was concluded that the investigated pectins can be easily extracted and successfully used as natural colorants or antioxidants since they have adequate antioxidant activity, total phenol and anthocyanin content and suitable color coordinates.

Keywords: pectin extraction, black currant, red currant, raspberry, blackberry, elderberry

1. Introduction

Solid agro-wastes formed in some fruit processing contain considerable amount of pectic substances which are complex, colloid carbohydrate polymers (Graham 1977) present in plant tissues (Munehiro et al. 2001) and are built up mostly by galacturonic acid residues (Walter 1991). Three different kinds of pectic polysaccharide structures (Hilz 2005) can be distinguished; homogalacturonate (HGA), rhamnogalacturonate I (RG I) and rhamnogalacturonate II (RG II).

Pectic substances are important compounds in the food, pharmaceutical and cosmetic industry (Walter 1992) since they are excellent gelling, thickening and emulsifying agents. Therefore realization of pectin extraction from agro-wastes is not only important from the environmental point of view but also enables the industry to achieve economical waste minimization and profitable production.

Furthermore in the case of colorful fruit processing some dying compounds can be extracted together with the pectin (Pantelidis et al. 2007). These colorants belong to the group of polyphenols (Lee and Finn 2007), which substantially contribute to the antioxidant complement (Pedisić et al. 2010) thus their presence in the recovered products has beneficial effects on the consumers (Wang et al. 2010). Even commercially available pectin is dyed by adding these coloring compounds in some cases, for example in the production of gel forming substances for special cakes (Schieber et al. 2003). Pectins obtained by extraction from vegetables and fruits (berries, apples, citrus fruits) are predominantly homogalacturonans and contain minor amounts of neutral side chains. The physical properties, such as gelling properties of commercial pectins depend on their molecular masses and on degree of esterification.

Although, the above-mentioned industries use citrus peels and apple pomace instead of pectin rich wastes as raw material for pectin production, intensive research work has been recently carried out on the recovery and

characterization of pectic substances from cocoa husks (Mollea et al. 2008), sunflower head (Sahari et al. 2003), peach pomace (Pagán et al. 2001), peels of mango (Berardini et al. 2005), banana (Emaga et al. 2008), ambarella (Koubala et al. 2008), and some other fruit waste (Madhave and Pushpalatha 2002). It was found that due to the heterogeneity of pectic polysaccharides their recovery was complicated. In most cases either the extraction by hot water followed by precipitation with ethyl alcohol (Hilz et al. 2005; Emaga et al. 2008) or the extraction by hot water were applied (Madhave and Pushpalatha 2002).

Unfortunately pectin extraction from agro-wastes formed by the processing of berry juices has been poorly studied due to the relatively lower pectin content of these fruits and the tough cell wall of polysaccharides found in black currant or in bilberries (Hilz et al. 2006).

Recovery of coloring, antioxidant molecules from colorful fruits is a more intensively studied field of research since black currant (Landbo and Meyer 2001), blue berry (Lee and Finn 2007) and several other berries (Pantelidis et al. 2007) has already been studied for this purpose and were suggested to be used as natural food colorants (Koroknai et al. 2008).

Combining the advantages of pectic substances and coloring, antioxidant compounds can be easily realized with the simultaneous extraction of colorants and pectins, which was reported only once with the application of apple pomace instead of pectin rich wastes (Schieber et al. 2003).

Therefore, the aim of this work was to study the simultaneous recovery of pectic substances and coloring, antioxidant compounds from press residues (cakes) of raspberry, blackberry, elderberry, red and black currant and to study the characteristics (color, antioxidant activity, total phenol and antocyanin content) of these compounds by the tristimulus color perception and FRAP methods.

2. Materials and Methods

2.1. Materials

Red currant (*Ribes rubrum*), black currant (*Ribes nigrum*), raspberry (*Rubus idaeus*), blackberry (*Rubus caesius*) and elderberry (*Sambucus nigra*) press residues, which were stored at -18 °C were provided by Fitomark '94 Ltd. (Tolcsva, Hungary) in 2008.

Purified apple (Classic AF 202) and citrus pectins were obtained from Herbstein & Fox, Germany and Poldin Ltd., Budapest, respectively.

Pectinex Ultra SP-L enzyme complex (from *Aspergillus aculeatus*) was purchased from Novozymes, Denmark.

All other chemicals were analytical grade and purchased from Fluka, Germany.

2.2. Extraction of pectic substances

Pectic substances were extracted from berry press residues by hot water, where the ratio of pectic substance and water were 1:4 (m/m %) in a 4 hours long process. As a result majority of pectic polysaccharides could be found in the aqueous phase, which was clarified and concentrated up to 5% TSS (total solid substance) by ultrafiltration using a polyethersulfone membrane with the cut off 45 kDa and then concentrated up to 30% TSS by evaporation. The pectin powder product was achieved by precipitation of the concentrated solution with ethyl alcohol.

2.3. Determination of the composition of pectic substances

Galacturonic acid (monomer) content of the pectic substances was measured in a total enzymatic hydrolysis process using Pectinex Ultra SP-L enzyme complex followed by HPLC analysis (column: Aminex HP 42, detector: RI 71, Merck).

Neutral monosaccharide content was determined by a concentrated trifluoroacetic acid (TFA) treatment for 2 hours at the temperature of 100 °C followed by HPLC analysis (Column: HP Zorbax Carbohydrate, detector: RI 71, acetonitrile and water mixture composition: 80:20 V/V %).

2.4. Color analysis

The International Commission on Illumination Standard L* (lightness), +a* (redness) and +b* (yellowness) color coordinates of pectins were determined by a HunterLab Miniscan spectrophotometric colorimeter, which is a compact analyzer for trans-reflectional color measuring in liquids and suspensions, applying cuvettes with internal diameter of 25 mm and sample layer of 10 mm. Standardization was realized by a standard-white reflection plate.

Values of L^* , a^* and b^* were determined to describe the three-dimensional color space where L^* indicated the lightness (in the range of 0-100 from completely opaque to completely white), a^* indicated redness and b^* yellowness on the hue circle. The confidence intervals for L^* , a^* and b^* were ± 3.5 , ± 0.9 and ± 0.8 , respectively. The hue angle calculated as $h = \arctg(b^*/a^*)$ expresses the color nuance (Voss 1992). The values are defined as follows; red-purple: 0° , yellow: 90° , bluish-green: 180° and blue: 270° (McGuire 1992).

The C^* chroma calculated as $(a^{*2}+b^{*2})^{1/2}$ is a measure of chromaticity, which indicates the purity or the saturation of the color. The color of pectin powders were measured by a Minolta tristimulus colorimeter on a surface with the diameter of 8 mm while pectin solutions were measured similarly on a surface with the layer thickness of 5 mm.

2.5. Determination of antioxidant activity, total phenol and anthocyanin content

The spectrophotometric method of Benzie and Strain (FRAP assay), which was developed to measure the ferric reducing ability of plasma at low pH using 2,4,6-tripyridyl-s-triazine and $FeCl_3$ reagents in pH 3.6 acetate buffer, was used to measure the antioxidant capacity of extracted pectins and fruit juice obtained in the concentration process (Benzie and Strain 1996). The absorbance was determined at 359 nm with the help of a calibration curve for ascorbic acid (AS) and the results were given as g (AS)/L or mg (AS)/L or g (AS)/g (sample) or mg (AS)/g (sample).

Total phenol content was measured by spectrometry at 760 nm using Folin-Ciocalteu reagent (Singleton and Rossi 1965). The amount of total phenolic content was estimated with the help of a standard curve of gallic acid (GA) and the results were given as g (GAE)/L or mg (GAE)/L or g (GAE)/g (sample) or mg (GAE)/g (sample), where GAE was the gallic acid equivalent.

Total anthocyanin content was determined at 510 and 700 nm in pH 10 (0.2 M solution of hydrochloric acid and potassium chloride) and pH 4.5 (1 M solution of acetic acid and sodium acetate) buffers by the pH differential absorbance method (Cheng and Breen 1991). The absorbance (A^*) was calculated by the following equation:

$$A^* = (A_{510} - A_{700})_{pH10} - (A_{510} - A_{700})_{pH4.5}$$

The anthocyanin content was estimated from the absorbance values with the help of a molar extinction coefficient of 29600 (cyanidin-3-glucoside) and a calibration curve. The results were given as g (cyanidin-3-glucoside equivalent)/L or mg (cyanidin-3-glucoside equivalent)/L or g (cyanidin-3-glucoside equivalent)/g (sample) or mg (cyanidin-3-glucoside equivalent)/g (sample).

2.6. Statistical analysis

All of the applied methods were investigated from the statistical analysis point of view. The Statistica 8.0 program was used for the evaluation of experimental data and confidential intervals were determined for each measurement methods. The experiments were carried out at least three times and the obtained results were subjects to analysis of variance and means using significance level of $p < 0.05$.

3. Results and Discussion

3.1. Extraction of pectic substances

Pectic substances were extracted from red currant, black currant, raspberry, blackberry and elderberry press cakes by the hot water extraction method. No pH adjustment was applied since preliminary experiments showed that the pH of the solution was in the advantageous acidic range (between pH 3.2 and pH 4.5) in all cases. The amount of alcohol insoluble solid pectin (AIS) obtained from 100 g initial fruit material and their pectin content (%) found in the literature both for fresh berries and press cakes are summarized in *Table 1*.

It can be seen that the pectin content data found in the corresponding literature are varying in a wide range thus it is quite difficult to compare them to the measured values. It is perceptible that the amount of measured pectin is lower in the case of press cakes than in the case of fresh berries for all fruits. It can be easily explained taking into account the fact that small amount of the pectic substances always leave with the fruit juice in the fruit processing steps.

Table 1. Data on extraction of pectic substances from various berries and berry press cakes

Substance	g AIS/ 100 g initial material	pectin content [%] (Walter 1992, Voss 1992, McGuire 1992, Vulic et al. 2008)
BC	1.4±0.4	0.20–1.79
BC press cake	1.1±0.3	No data
RC	1.3±0.3	0.91–1.5
RC press cake	0.95±0.2	No data
R	1.0±0.3	0.58–1.86
R press cake	0.9±0.4	No data
B	1.4±0.5	0.68–1.89
B press cake	1.2±0.3	No data
E	3.1±0.6	3.96
E press cake	2.9±0.6	No data

3.2. Determination of the composition of pectic substances

The galacturonic acid and monosaccharide content of the various berry pectins were determined and are presented in Table 2. For comparative purposes not only berry pectins but the commercially available citrus pectin was studied, as well.

Table 2. Data on galacturonic acid and monosaccharide content

Mass%	Pectins		
	BC press cake	RC press cake	C
Galacturonic acid	37.1±7.2	49.7±7.9	50.3±8.2
Xilose	7.4±2.3	0.2±0.05	1.2±0.4
Arabinose	5.7±1.9	2.3±0.7	8.7±2.1
Mannose	15.4±3.4	16.0±4.2	11.9±2.3
Glucose/galactose	31.4±7.5	25.3±7.1	25.5±6.4

Galacturonic acid content varies in a wide range according to the literature data, 46 to 78% for citrus pectin (Iglesias and Lozano 2004) or 20 to 50% for black currant pectin (Hilz et al. 2005). The results of our experiments fall into the presented range (*Table 2*). It is shown that the galacturonic acid content is lower in the investigated berry press cakes than in citrus pectin and that the acid content of red currant press cake is higher than of black currant.

In the corresponding literature monosaccharide content of pectins is available only in the case of black currant in which glucose (20 mol %), mannose (13 mol %), arabinose (11 mol %), xilose (6 mol %) and galactose (6 mol %) are present and the rhamnose and fucose content are negligible. Similar values were determined in our experiments for mannose and xilose. It can be seen that the mannose content is higher and the arabinose content is lower than in the citrus pectin for both red and black currant. It was quite difficult to determine the amount of glucose and galactose since epimerisation might occur between these two compounds in the anion exchange column of the HPLC during the analysis. Therefore only the sum of these two polysaccharides was given in *Table 2*. Rhamnose and fucose were not found in the samples.

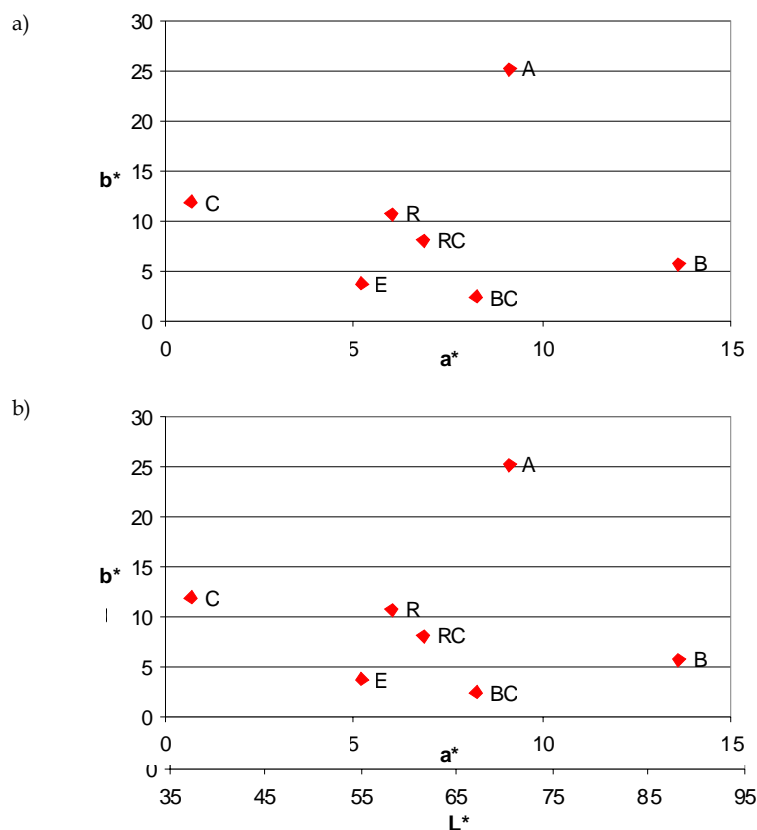
3.3. Color analysis

Color coordinates (L^* , $+a^*$, $+b^*$) were determined and values of C^* were calculated for all of the investigated pectins extracted from the berry press cakes and for commercially available citrus and apple pectins. Pectin powder was obtained from the pectin extracted firstly, then 1% aqueous solution and gel (with added sucrose) were prepared. These materials represent the main possible practical applications.

The color coordinates of pectin powders are presented in (*Figure 1a*) (C^* versus L^* values) and (*Figure 1b*) ($+b^*$ versus $+a^*$ values)[‡]. It can be seen (*Figure 1a*) that relatively low L^* and C^* values belong to pectin powders from blackberry, black currant, elderberry and raspberry, while citrus and red currant pectin powders have low C^* and high L^* values and apple pectin as high C^* and a bit lower L^* values. It is perceptible (*Figure 1b*) that black currant, elderberry, raspberry and red currant pectins have relatively

[‡]The abbreviations A, B, BC, C, E, R and RC stand for apple, blackberry, black currant, citrus, elderberry, raspberry and red currant pectins, respectively.

low $+a^*$ and quite low $+b^*$ values, blackberry pectin has an extremely high $+a^*$ and a quite low $+b^*$ value, citrus pectin has very low $+a^*$ and quite low $+b^*$ values and relatively high $+a^*$ and high $+b^*$ values belong to apple pectin.

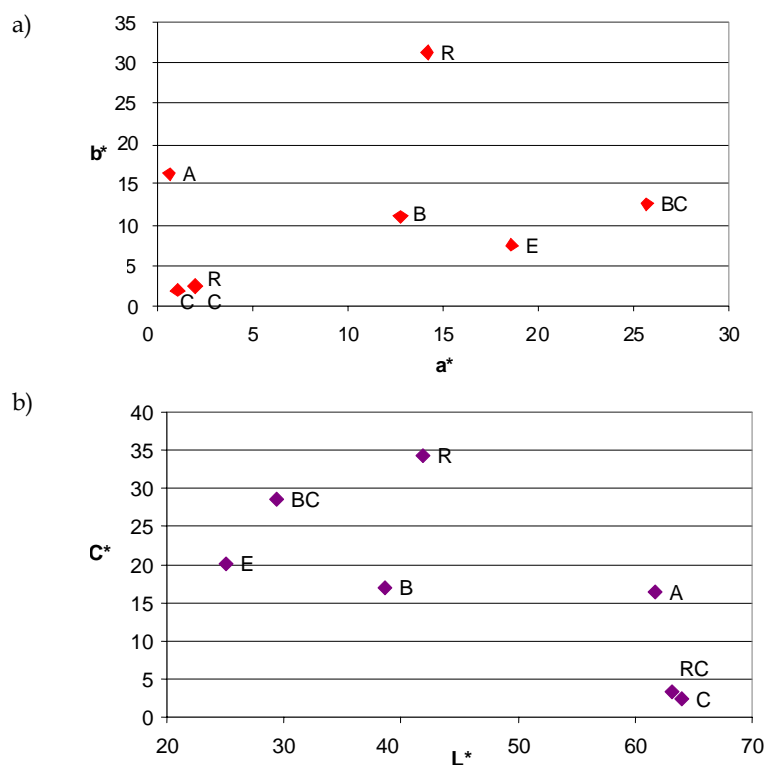


Figures 1a and 1b. Color coordinates of pectin powders from various berry press cakes, apple and citrus (abbreviations: A – apple, B – blackberry, BC – blackcurrant, C – citrus, E – elderberry, R – raspberry and RC – red currant)

This means that the pectin powders from black currant, raspberry, black berry press cakes have low lightness and chroma in the dark grey and dark brown region. The red currant, citrus and apple pectin powders, on the other hand, have high lightness and low chroma.

Figure 2a and Figure 2b represent the color coordinates of the 1% aqueous pectin solutions prepared from the previously studied fruit pectins and

Figure 3 shows the perceptible difference in the colors of the various pectin solutions.



Figures 2a and 2b. Color coordinates of solutions of pectin from various berries, apple and citrus

It is interesting to notice that in the case of pectin solutions the L^* values of elderberry, black currant, citrus, red currant pectins, the C^* values of apple, red currant, citrus pectins and the $+a^*$ and $+b^*$ values of the apple pectin decreased, while the C^* values of black currant, elderberry, blackberry, raspberry, black currant pectins, the $+a^*$ values of black currant, elderberry, raspberry and the $+b^*$ values of blackberry, black currant, raspberry pectins increased and the color coordinates of the other pectins remained the same compared to the results for pectin powders.

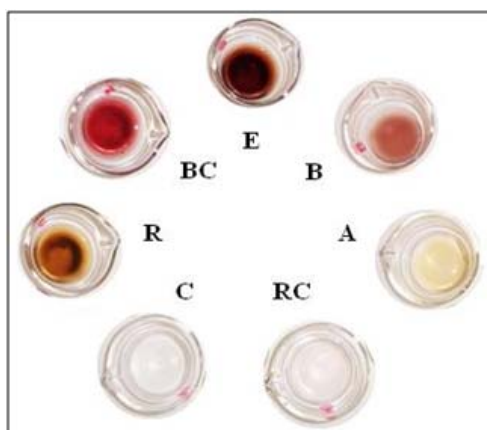


Figure 3. Colors of 1% pectin solutions made of various sources

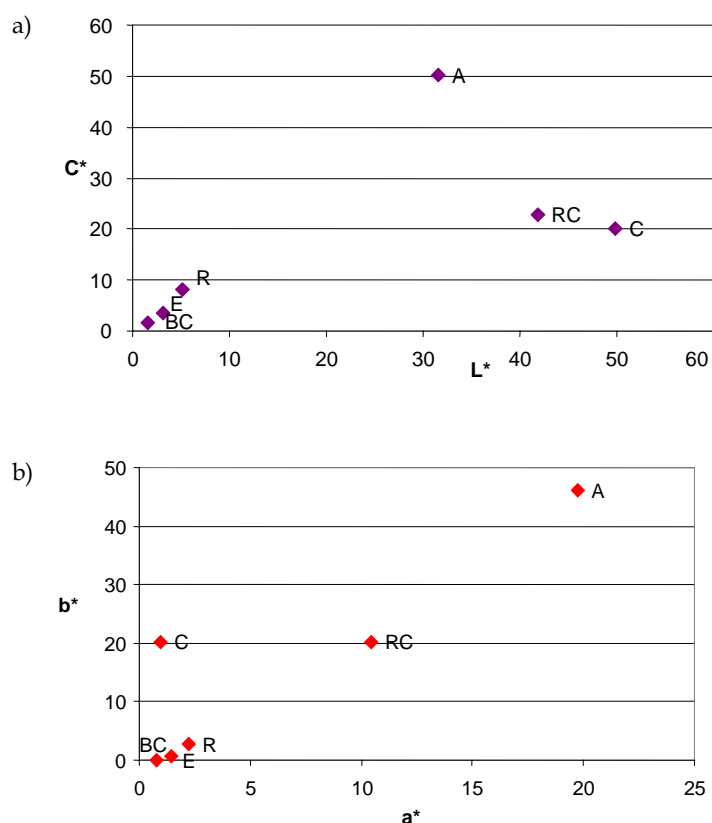
The color coordinates of pectin gels are represented in *Figure 4a* and *Figure 4b*. It can be noticed that pectin gels of the investigated fruits have similar L^* and C^* values as the pectin powders and that the $+a^*$ and $+b^*$ values increased in the case of red currant and apple pectin gels, decreased in the case of black currant, elderberry, raspberry pectin gels and remained the same for citrus pectin gel.

As a summary of the color analysis the hue angles of the investigated pectin powders, aqueous solutions and gels were calculated and are presented in *Table 3*.

Table 3. Hue angles (h_{ab}) for pectic substances from various berry press cakes, apple and citrus

Pectin	h_{powder}	h_{solution}	h_{gel}
BC press cake	16.82	26.06	2.12
E press cake	36.54	21.96	27.95
R press cake	60.79	65.48	51.56
RC press cake	49.70	51.28	62.62
B press cake	23.04	41.04	–
C	86.68	62.99	87.30
A	70.14	87.65	66.82

The results clearly show that the pectin colors fall in the range between reddish purple and yellow. Black currant and elderberry pectins can be characterized by the reddish purple feature, while the citrus and apple pectins are rather yellow.



Figures 4a and 4b. Color coordinates of pectin gels from various berries, apple and citrus

Furthermore it can be stated that some of the pectins in a powder form have different color coordinates than in the form of aqueous solutions or gels. This might be very important when the pectins are selected for different production processes.

3.4. Determination of antioxidant activity, total phenol and anthocyanin content

The results of the experiments concerning the antioxidant activity, total phenol and anthocyanin content of red and black currant pectins and juices are represented in *Table 4*. Comparing our results to the data found in the corresponding literature for red currant (Parrado et al. 2007) and black currant juice (Vulic et al. 2008) it can be seen that for black currant juice much lower antioxidant capacity and total phenol content and almost the same total anthocyanin content and for red currant much lower antioxidant capacity and higher total phenol and total anthocyanin content were given by our measurements.

Table 4. Values of antioxidant capacity, total phenol and anthocyanin content

Sample	Antioxidant capacity (FRAP)	Total phenol content	Total anthocyanin content
BC juice (Fuchs and Wretling 1991)	21.76 mmol AS/l (4.0 g AS/l)	2.5 g GAE/l	1.79 g/l
BC juice	1.1±0.2 g AS/l	0.7±0.1 g GAE/l	1.38±0.1 g/l
BC press cake pectin	32±2.1 mg AS/g	35± mg GAE/g	0.4 ±0.08 mg/g
RC (Sahari et al. 2003)	60.2–63.3 µmol AS/g dry weight (11.1–11.7 g AS/g)	1.1–1.2 g GAE/100 g dry weight	7.5 mg/100 g fruit
RC juice	240±14 mg AS/l	0.67±0.05 g GAE/l	430±25 mg/l
RC press cake pectin	90±7 mg AS/g	80±6 mg GAE/g	0.4±0.08 mg/g

It can be also noticed that the antioxidant activity and anthocyanin content of blackcurrant juice are much higher than that of red currant, while total phenol content is similar in both cases. For the pectin preparation total anthocyanin content is similar to red and black currant pectins and higher total phenol content belongs to the pectin extracted from red currant press cake. However, the anthocyanin content of pectin preparations are lower than the values of fruit juices. It can be concluded that the colorants found

in pectin preparations belong to the group of phenolics and have adequate antioxidant capacity, which is extremely beneficial for the human health.

Regarding the experimental results it can be concluded that majority of the investigated pectins are quite colorful containing natural colorants (pigments), which belong to the group of phenolics and have antioxidant capacity. This means that not only they can be used as natural colorants but they can have advantageous health effects on the consumer of the products prepared with these pectins.

Therefore, the recovery of these colorful pectins and their application in the food industry can contribute to the simultaneous economical production of value-added compounds and the environmentally sound utilization of solid agro-wastes.

4. Conclusions

In this work pectic substances were extracted from red currant, black currant, raspberry, blackberry and elderberry press residues by hot water and the composition, antioxidant activity, total phenol, anthocyanin content and the color coordinates of pectins were determined.

The experimental results show that the pectin colors fall in the range between reddish purple (black currant and elderberry) and yellow (citrus and apple). Moreover some of the pectins in powder form have color coordinates different from the forms of aqueous solutions or gels. This might be very important when the pectins are selected for different production processes. The anthocyanin content of pectin preparations is lower than the values of fruit juices. It can be concluded that the colorants found in pectin preparations belong to the group of phenolics and have adequate antioxidant capacity, which is extremely beneficial for human health.

The investigated pectins can be easily extracted and successfully used as natural colorants or antioxidants in the forms of either powder, or solution or gel, since they have adequate antioxidant activity, total phenol and anthocyanin content and suitable color coordinates.

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